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BIOCHEMISTRY OF WILTED TOMATO PLANTS

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ABSTRACT:

Tomato (*Solanum lycopersicum*) is one of the widely grown vegetables worldwide. *Fusarium oxysporum* f. sp. *lycopersici* (*FOL*) is the significant contributory pathogen of tomato vascular wilt. Vivotoxins like fusaric acid and lycomarasmin designate as secondary metabolite produced by the pathogen and/or its host during infection, produces disease symptoms, but is not oneself the initiating causal agent of the infection. Out of two, Fusaric-acid is the most studied pathogen produced wilt toxin classified as a non-specific vivotoxin. It does not produce all the symptoms of wilt. Many scientists all over the world including India were of the belief that, out of all other toxins involved in the infection process of wilting, fusaric acid was the most potent one. Infected tissue shows a marked increase in respiration process which is contrary to the host tissues doped with fusaric acid because it is a best-known respiratory depressant.

KEYWORDS: *Fusaric-acid, Fusarium oxysporum, Potent, Vivotoxin, Wilting.*

INTRODUCTION:

Tomato (*Solanum Lycopersicum)* is the second most economically important vegetable crop cultivated throughout the world. It has

immense nutritional value and antioxidant properties (Nahar and Ullah., 2012). The crop is susceptible to over 200 plant diseases of which mostly are of fungal in nature. In field condition, yield of tomato is severely hampered by wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc) Snyder et Hansen (Agrios., 2005). Differentiating between *Fusarium* strains is very difficult to realize because of its diversity in species and formae speciales (Benlamoudi et al. 2014). The initial symptoms of the disease appear in the lower leaves gradually, trail by wilting of the plants. It has been reported that FOL penetrates the tomato plant, colonizing and leaving the vascular tissue dark brown, and this discoloration extends to the apex, leading to the plants wilting, collapsing and dying. Therefore, it has been widely accepted that wilting caused by this fungus is the result of a combination of various physiological activities, including the accumulation of fungal mycelia in and around xylem, mycotoxin production, inactivation of host defense, and the production of tyloses; however, wilting symptoms are variable (Srinivas et al. 2019).

Dimond and Waggoner, 1953 gave the term "vivotoxin" to denominate "a substance produced in the infected host by the pathogen and/or its host, which functions in the production of disease, but is not itself the initial inciting agent of disease." They also notified that "a vivotoxin is a disease-producing entity and therefore a pathogenic agent." They enlisted 3 major criteria as the nominal need to set up vivo toxicity. These were -

(a) reproducible segregation from the infected host plant,

(b) purification, and

(c) re-production of at least a fraction of the disease symptoms by allocating the toxin in a same healthy plant.

Dimond, 1955 subsequently altered their criteria by defining that vivotoxin "not be present in the healthy host" because it produces during the host-pathogen interaction. Dimond as well explained that, instead of refinement, the toxin be defined chemically. Dimond & Waggoner seemed to be cognizant of this in consideration that vivo toxicity could be incontestable by observing only the 1st and 3rd of their criteria. Paradoxically, they said that "just as it is usually necessary to know the identity of a parasite to establish it as a cause of disease, so it is also necessary to purify and identify a vivotoxin to prove its complicity." We disagree with the postulate expressed in the first section or with the assumption attained in the last. What is required in both the cases is indicated in the conclusion that the parasite or the toxin perform a significant causative function during the occurrence of biotic infection. Here we want simply signalize that criteria which stipulate the requisite evidence are more adequate than those which

prescript the processes by which the information is to be received. First step of Dimond & Waggoner's, which shows segregation of the toxin from the infected host plant, is based on the aforementioned criticism. Separation of most potent, coseismal toxin, existing in little bulk, seems impracticable, but tolerable illustration is that it utilizes in the creation of infection symptoms may be acquired by some different ways (Braun and Pringle, 1959). Despite the fact that these considerations betoken that the criteria of Dimond & Waggoner are little idealistic because they don't explain straight on the query of their cogency.

Two different low molecular weight toxins have been found in *Fusarium* Cultures and both have been implicated as wilting agents in the tomato. They are the nitrogen containing lycomarasmin and a pyridine derivative fusaric acid (5-*n*-butylpicolinic acid). The role of lycomarasmin in pathogenicity is still not entirely clear because although readily formed in culture, it has yet to be detected unequivocally in infected plants. This may be simply due to its great lability in solution. An interesting point regarding the mode of action of these pathotoxins is that both lycomarasmin and fusaric acid are metal chelators. The former has strong chelating properties and its translocation and activity may be related to the water-soluble complex it forms with iron. The activity of fusaric acid is also related to the metal ion content, Since its production, at least in culture, is dependent on the presence of zinc and unless sufficient is added, its synthesis is depressed. The role of fusaric acid in causing wilting is reasonably well established.

Many scientists all over the world including India were observed that, even the presence of many other toxins during the infection process of wilting, fusaric acid was the highest powerful one and put forward the pursuing grounds to explicate why fusaric-acid didn't produce symptoms during the initiation of infection process:

(a) fusaric acid alienates at above 6.0 pH and the pH of infected host plants sap during initial execute of infection process is 6.2;

(b) fusaric acid is produced in very little amounts during infection initiation (Subba-Rao, 1960).

Lycomarasmin: It is exclusively produced by *Fusarium oxysporum* f.sp. *lycopersici* causing tomato wilt. Gaumann and co-workers (1952) have isolated lycomarasmin and fusarinic acid as toxins produced in the culture filtrates of the tomato wilt fungus. Symptoms of tomato wilt simulate the toxic injury manifested by the synergistic action of lycomarasmin and fusaric acid. They also reported another wilt toxin vasofuscarin which is also claimed to play a role in disease development (Gaumann *et al.* 1953). Lycomarasmin is a polypeptide with molecular weight 277.3 and empirical formula - $C_9H_{15}O_7N_3$. Woolley (1948) postulated lycomarasmin to be the modified tripeptide as shown in figure1:-

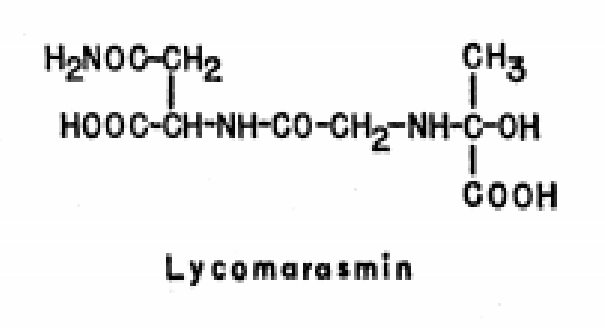

Figure 1 – Lycomarasmin structure

The toxin inactivates the growth factor strepogenin. If this growth factor is applied artificially to the infected plants symptoms are reduced. Purified lycomarasmin acts as a chelating agent and after application to tomato cuttings it forms a water soluble unstable chelate with iron which is translocated to leaves (Waggoner and Dimond, 1953). Iron is set free in the leaves and causes typical symptoms. Iron increases toxicity of the toxin while copper reduces it. In the presence of iron, lycomarasmin causes increased transpiration of tomato cuttings and increased water permeability of epidermis (Ludwig, 1960).

Fusaric-acid

This secondary metabolite was first reported in 1934 from *Fusarium heterosporum*, but its toxic nature was recognized about two decades later by Gauman et al. in the year 1952, who also reported its occurrence from *Fusarium oxysporum* f.sp. *lycopersici*, *Fusarium oxysporum* f. sp. *vasinfectum* and *Gibberella fujikuroi*. Since then this phytotoxic metabolite has been detected in various *Fusarium* formae specialis of the elegance group which included *Fusarium oxysporum* f.sp. *lycopersici*, *batatis, conglutinans, cubense, lini, vasinfectum, udum* and *Fusarium moniliforme* (Gaumann, 1957; Kalyanasundaram, 1958; Heitefuss *et al.* 1960a, 1960b; Trione, 1960a, 1960b; Prasad and Chaudhary, 1974).

It is the most studied pathogen produced wilt toxin classified as a non-specific vivotoxin. It does not produce all the symptoms of wilt. Fusaric acid is a pyridine-carboxylic acid having empirical formula $C_{10}H_{13}O_2N$ (figure 2)and chemically this toxin is 5-n-butyl-picolinic acid.

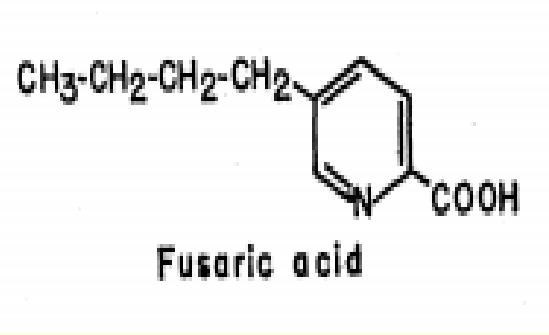

Figure 2: Fusaric acid

Biosynthesis of fusaric acid is accomplished even in synthetic media, which shows that no additional nutrition is required for its production. However, it has been noticed that its production is conditioned by the amount of zinc present in the culture medium. Kalyanasundaram and Saraswathi Devi (1955) noted that secretion of fusaric acid by *Fusarium oxysporum* f.sp. *vasinfectum* required 0.08 - 0.4 ppm of zinc (Zn), the optimum concentration being 0.24ppm. Prasad and Chaudhary (1974) also reported stimulatory influence of zinc on fusaric acid production by *Fusarium oxysporum* f.sp. *udum*.

It is now well recognized that fusaric acid is produced during the rapid growth phase, and is not a product of autolysis (Sandhu, 1960). Its synthesis seems to be linked with the intermediates of Krebs cycle and is a primary metabolite. Although growing hyphae secrete fusaric acid, most of it is liberated after mycelial autolysis starts (Bohni, 2016). This metabolite has also been detected in the mycelial extracts of different strains of *Fusarium oxysporum* (Prasad and Chaudhary, 1974), which indicates that the entire quantity synthesized by the fungus is not secreted out, rather some of it is retained in the hyphae.

Synthesis of fusaric acid *in vivo* has also been studied and some isotopic data were obtained to demonstrate its production in the tissue of the host-plant (Kern and Sanwal, 1954; Kern and Kluepfel, 1956). Direct detection of this metabolite in the tissue-extract of diseased host plants has also been attempted and met with appreciable success (Lakshminarayanan and Subramanian, 1955; Kalyanasundaram and Venkata Ram, 1956).

Production of the toxin by some species in the rhizosphere soil of tomato plant is also reported (Kalyanasundaram, 1958). In contrast, there are certain reports (Heitefuss et al. 1960a), according to which no fusaric acid is produced in the host tissues, although the same pathogen produces fusaric acid in culture solution. They further concluded that this toxin had apparently no role in pathogenicity. Kuo and Scheffer (1964) have also doubted the role of fusaric acid in disease development, and full details are not clearly understood.

The toxin is active at 20-200mg/kg fresh weight. Sometimes, another toxin, dehydrofusaric acid is associated with fusaric acid which is easily converted into the latter. It mainly causes interveinal chlorosis. The role of fusaric acid in the plants is said to be of many types. It causes chelation of iron and copper in the host cells and alters the cell wall permeability. This disturbs the ionic balance of the cell. It also affects the enzymatic processes in the cell. By chelating the enzymes or by rendering respiratory enzymes ineffective, it alters respiratory pattern of the plant. However, whether fusaric acid is responsible for causing all the symptoms in the diseased

host plant infected with wilt-fusaria is yet to be established because wilt syndrome in plants is produced by a combination of several toxins and metabolites.

This secondary metabolite is also toxic to bacteria, algae, fungi and angiospermic plants. Some of its notable effects are in Table 1.

Fusarium wilt is one of the most prevalent and damaging diseases of tomato. Among various toxins secreted by the *Fusarium oxysporum* f. sp. *lycopersici* (causal agent of Fusarium wilt of tomato), fusaric acid (FA) is suspected to be a potent pathogenicity factor in tomato wilt disease development. With this rationale the present study was carried out with physiological, biochemical and proteomic perspectives. Treatment of FA was given to the leaves of tomato directly through infiltration to show the characteristic features of Fusarium wilt of tomato. The phytotoxic effect of FA was assessed in the form of cell death in tomato leaves which was observed by increased uptake of Evans blue stain. The measurement of electrolyte leakage was used as an indicator of the extent of cell death. The influence of FA on the leaf photosynthesis of tomato plant was investigated and it was found that FA strongly reduced the photosynthetic pigment contents of tomato leaves resulting to heavy suppression of leaf photosynthesis processes, which therefore affected leaf physiology finally leading to leaf wilting and necrosis. This cell death inducer (FA) produced an enormous oxidative burst during which large quantities of reactive oxygen species (ROS) like H2O2 was generated in the treated leaf tissues of tomato plants which was evident from enhancement in lipid peroxidation. To assess the involvement of proteolysis in the cell death cascade induced by FA treatment, total protease activity was measured in the leaf tissues and it was found that the total protease activity increased with the treatment and leading to cell death. Furthermore, proteomic study was used as a powerful tool to understand the alterations in cellular protein expression in response to FA exposure. Differential expression in several proteins was observed in the present study. Proteomic analyses, thus, clearly indicate that proteins belonging to different functional classes are significantly affected in the plant leaf tissues after FA exposure leading to deterioration of structure and metabolism of cells. Thus, it is concluded that FA plays an important role in fungal pathogenicity by decreasing cell viability (Singh et al. 2017).

CONCLUSION:

Tomato is one of the important and widely grown vegetable crop in the world. Plant diseases are one of the major constraints in tomato production. Vascular wilt disease in tomato is one of them. Vivotoxins like fusaric acid and lycomarasmin designate as secondary metabolite produced by the pathogen and/or its host during infection, produces disease symptoms, but is not oneself the

initiating causal agent of the infection. Out of two, Fusaric-acid is the most studied pathogen produced wilt toxin classified as a non-specific vivotoxin. It does not produce all the symptoms of wilt. Many scientists all over the world including India were of the belief that, out of all other toxins involved in the infection process of wilting, fusaric acid was the most potent one. Infected tissue shows a marked increase in respiration process which is contrary to the host tissues doped with fusaric acid because it is a best-known respiratory depressant.

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Table 1: Showing toxic effects of Fusaric-acid on various microbes and plants

Organisms	Effect	Concentration
Bacteria	Growth inhibited	10^{-4} to 10^{-3} M
Green Algae *Spirogyra nitida*	Permeability affected	5 x 10^{-3} M
Ustilago maydis	Germination of basidiospores effected	1.5 x 10^{-4} M
Rye, maize and pea plants	Injury caused	1.000 to 2000 mg/Kg fresh weight
Tomato plants	Injury caused	150 mg/Kg fresh weight
Cotton plants	Injury caused	10 to 20 mg/Kg fresh weight